b.) Remarks

Claims 1-9, 13-16 and 28-30 have been amended in order to recite the invention with the specificity required by statute and claims 34-48 are added in order to more specifically recite preferred embodiments of the present invention. No new matter has been added.

Claims 22-27 and 32 are rejected under 35 U.S.C. §101 because the claims of "use" claims are not directed to statutory subject matter. Claims 20 and 33 are also rejected under 35 U.S.C. §112, first paragraph, because the specification does not provide written description for PDE 10A inhibitors outside of formula I, IA or compounds of prior art known to have PDE 10A inhibition activity. These rejections are addressed by the foregoing cancellation of those claims.

Claims 17-20, 28-31 and 33 are rejected under 35 U.S.C. §112, first paragraph, as lacking of written description and enablement because there is no adequate description showing the nexus between the inhibition of PDE 10A and a useful treatment of a disease or condition, or working example(s) of compounds having PDE 10A inhibition activity used for treating or preventing disease. This rejection is respectfully traversed.

PDE 10A is one of the PDE subtypes and has the activity to hydrolyze cGMP to GMP. Though the subtypes have been classified according to biochemical characteristics, enzymological characteristics and the like, the common activity of PDE is hydrolyzation of cyclic nucleotide which is known as an important second messenger in cells. Therefore, it is also well-known that there is a relationship between PDE activity and disease or condition in mammal. For example, one form of diabetes insipidus in the mouse has been associated with increased phosphodiesterase Family 4 activity and an

increase in low-K, cAMP phosphodiesterase activity has been reported in leukocytes of atopic patients. It is also known that defects in cyclic nucleotide phosphodiesterases have been associated with retinal disease. Further, Family 3 phosphodiesterase has been associated with cardiac disease.

Moreover, cyclic nucleotide phosphodiesterases have also been reported to affect cellular proliferation in a variety of cell types and are implicated in the treatment of various cancers. Bang et al., (*Proc. Nat. Acad. Sci.*, USA, Vol. 91 (1994) 5330-34) teach that a permanent conversion in phenotype from epidermal to neuronal morphology is observed by delivery of cAMP and phosphodiesterase inhibitor and Deonarain et al. (*Brit. J. Cancer*, Vol. 70 (1994) 786-94) suggest a tumor targeting approach to cancer treatment that involves intercellular delivery of phosphodiesterases to particular cellular compartments, resulting in cell death.

On the other hand, PDE 10A operates in putamen, caudate nucleus regions of brain and testis, striatum, nucleus accumbens and olfactory tubercule in view of Fujishige et al. (*J. Biol. Chem.*, Vol. 274 (1999) 18438)[1], WO 2001/24781[2] and general knowledge[3]. Further, Example 13 in WO 2001/024781 shows that PDE 10A modulator is effective for Huntington's disease. Therefore, it is highly expected that the activity of PDE 10A affects to neuronal disease in the above regions and diseases concerning cell proliferation such as cancer.

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[1] Fugishige teaches that human PDE 10A is expressed in human fetal brain and PDE 10A transcripts are particularly abundant in the putamen and caudate nucleus regions of brain and testis.

[2] Example 10 shows that expression of PDE 10A mRNA was restricted to the striatum, nucleus accumbens and olfactory tubercle.

[3] Proteins or receptors are usually thought to have some function in the region having high RNA or protein expression.

As to working examples in the present specification, Applicants shows that the compounds of formula I inhibits hyperkinesias in 6-OHDA-treated rats, which is a recognized model of diskinesia which is a side effect induced by long-term administration of L-DOPA as a therapeutic agent for Parkinson's disease (see page 53, lines 12-17 and the abstract of *Exp. Neurol*, Vol. 151 (1998), 334-42, attached). Test Example 3 shows that the compounds of formula I inhibits the growth of human breast cancer cells.

Claims 1-11, 15-21, 28-31 and 33 are rejected under 35 U.S.C. §102(a) or (b) as anticipated by Pospil (CA 138:283178, *J. Receptor and Signal Transduction*, Vol 22, Nos.1-4 (2002) 141-54). Claims 20 and 33 are rejected as being anticipated by WO 95/17904, and claims 1, 3, 20, 28 and 33 are rejected as being anticipated by WO 2001/77110 and Gomez-Parra. This rejection is respectfully traversed.

Of course, the rejection over WO 95/17904 is mooted by the cancellation of claims 20 and 33. Further, neither Posil nor WO 2001/77110 teach the method for inhibiting PED 10A or for treating a disease caused by enhancing the activity of PDE10A by administration of the compound (I). Finally, amended claim 9 does not encompass any compounds taught or suggested by Pospil.

Claims 12-14 are noted to recite patentable and unobvious subject matter and are objected to solely as depending from a rejected base claim. The Examiner's assistance and cooperation in expediting the prosecution of this application by examining separately the subject matter of Applicants' dependent claims is gratefully acknowledged.

In view of the above amendments and remarks, Applicants submit that all of the Examiner's concerns are now overcome and the claims are now in allowable condition.

Accordingly, reconsideration and allowance of this application is earnestly solicited.

Claims 1-16, 28-31 and 34-48 remain presented for continued prosecution.

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Characterization of Enhanced Behavioral Responses to L-DOPA Following Repeated Administration in the 6-Hydroxydopamine-Lesioned Rat Model of Parkinson's Disease

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Long-term treatment of Parkinson's disease with dopamine-replacing agents such as L-3,4-dihydroxyphenylalanine (L-DOPA) is compromised by many side-effects, most notably involuntary movements, L-DOPA-induced dyskinesia. Acute challenge with dopamine-replacing drugs elicits a rotational response in the 6-hydroxydopamine (6-OHDA)-lesioned rat model of Parkinson's disease. This rotation is contraversive to the lesion and is considered to represent an antiparkinsonian effect. More recently, it has become clear that the rotational response shows plasticity and that repeated L-DOPA or apomorphine therapy is accompanied by a marked enhancement in this response. In this study, we demonstrate that the enhanced behavioral response to repeated dopamine-replacement therapy seen in the 6-OHDA-lesioned rat has pharmacological characteristics similar to L-DOPA-induced dyskinesia seen in MPTP-lesioned primates and man. Thus, the magnitude and rate of development of the enhanced response to L-DOPA treatment is related to both the number of doses and the size of the dose of L-DOPA administered. In contrast, *de novo* administration of dopaminergic drugs that are associated with a lower incidence of dyskinesia, e.g., bromocriptine or lisuride, does not lead to an enhanced behavioral response following repeated treatment. However, following a single "priming" administration of apomorphine, the rotational response elicited by subsequent bromocriptine administrations is enhanced with repeated treatment. Once established, the enhanced behavioral response to repeated L-DOPA-administration (6.5 mg/kg, twice daily) can, like L-DOPA-induced dyskinesia in man and MPTP-treated monkeys, be selectively reduced by coadministration of L-DOPA with the alpha$_2$-adrenergic receptor antagonist yohimbine (10 mg/kg, −95%), the 5-HT uptake inhibitor 5-MDOT (2 mg/kg, −90%), or the beta-adrenergic receptor antagonist propranalol (10 mg/kg, −35%). While these rats do not exhibit symptoms of dyskinesia per se, this rodent model does exhibit behaviors, the underlying mechanism of which is likely to be similar to that underlying L-DOPA-induced dyskinesia and may prove useful in studying the molecular and cellular mechanisms of L-DOPA-induced dyskinesia in Parkinson's disease.

Key Words: **Parkinson's disease; L-DOPA-induced dyskinesia; 6-hydroxydopamine.**

INTRODUCTION

For over 30 years the dopamine precursor L-3, 4-dihydroxyphenylalanine (L-DOPA) has been widely employed in the symptomatic treatment of Parkinson's disease (14, 40). In the initial years of treatment, L-DOPA is highly successful in reversing the parkinsonian symptoms of hypokinesia, bradykinesia, tremor, and rigidity. However, following several years of treatment many complications are observed. The most common of which are the abnormal involuntary movements termed L-DOPA-induced dyskinesia (2). Following 5 years of antiparkinsonian therapy with L-DOPA, up to 80% of patients develop symptoms of L-DOPA-induced dyskinesia (28). These dyskinesias can eventually become as debilitating as the Parkinson's disease itself (28, 36). Conversely, *de novo* administration of certain long-acting D2 dopamine receptor agonists, such as bromocriptine or lisuride, is associated with a lower incidence of dyskinesia in either patients or 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP)-lesioned macaques (3, 9, 24). Unfortunately, only about 30% of patients respond well to the antiparkinsonian effects of bromocriptine (25) and patients who receive bromocriptine monotherapy may also experience adverse effects, including confusion, visual hallucinations, and paranoia (8, 21). Therefore, the majority of patients require bromocriptine plus L-DOPA or L-DOPA monotherapy, both of these regimens eventually resulting in dyskinesia (23, 26, 29, 34). Similarly, once MPTP-primates have been primed to elicit dyskinesia in response to

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challenge by L-DOPA or apomorphine subsequent challenge with bromocriptine elicits dyskinesia (4, 31). In a similar manner, *de novo* administration of lisuride has also been shown to be associated with a low incidence of dyskinesia (35). However, following priming by dopamine-replacement therapy, administration of lisuride, or its semisynthetic analogue terguride, elicits dyskinesia (4, 6). It is unclear at present how well *de novo* treatment of parkinsonian symptoms with the new generation of dopamine receptor agonists (ropinirole, cabergoline, and pramipexole) will provide relief of symptoms in the long term (33, 1, 27). However, studies in the MPTP-treated primate suggest that, if given to patients previously primed to elicit dyskinesia with L-DOPA, they will also elicit dyskinesia (4, 6).

The neural mechanisms underlying L-DOPA-induced dyskinesias remain largely unknown. Given the complex nature of the interactions between many factors thought to be responsible for the generation of L-DOPA-induced dyskinesia, it is difficult to perform well-controlled incisive clinical studies. Thus, to elucidate the neural mechanisms underlying L-DOPA-induced dyskinesia, investigate and develop effective adjunct therapies for L-DOPA-induced dyskinesia, and screen for novel antiparkinsonian drugs that do not cause dyskinesia, the most valuable asset is an effective animal model. To date, the only animal model which replicates the symptomatic changes of L-DOPA-induced dyskinesia is the MPTP-lesioned nonhuman primate following repeated L-DOPA or dopamine-agonist administration (3, 7, 12, 31, 20). This is an excellent model in almost all respects and will undoubtedly remain the "gold standard" for the investigation of L-DOPA-induced dyskinesias. However, part of the reason for the slow rate of advance in our understanding of the mechanisms underlying L-DOPA-induced dyskinesia arises from logistical difficulties in using this model. Furthermore, ethical considerations must lead us to consider nonprimate models that may permit useful advances toward understanding the neural mechanisms underlying L-DOPA-induced dyskinesia. Therefore, a rodent model that replicates the biochemical, molecular, and neural mechanisms underlying L-DOPA-induced dyskinesia would be of critical importance. To date, no rodent model of L-DOPA-induced dyskinesia has been validated. Indeed, given the repertoire of behaviors exhibited by rodents it is probably unlikely that such species could display movements with the complexity to be considered as obvious counterparts to symptoms such as chorea, dystonia, and athetosis seen in primates.

It is widely known that dopamine replacement therapy elicits rotational behavior in the 6-hydroxydopamine (6-OHDA)-lesioned rat model of Parkinson's disease (38, 39). The direction of this rotation is contraversive to the side of the lesion and is generally considered to represent an antiparkinsonian effect (39). However, several authors have described that the response exhibits marked plasticity such that, following repeated treatment, an enhancement of contraversive rotation induced by either L-DOPA or apomorphine is seen (5, 10, 16).

In this study, we characterize the pharmacology of this enhanced behavioral response to repeated dopamine replacement therapy in the 6-OHDA-lesioned rat and compare it to that of L-DOPA-induced dyskinesia seen in the clinic and in the MPTP-lesioned nonhuman primate. The effects of *de novo* treatment with L-DOPA, bromocriptine, or lisuride are compared. Additionally, we investigate the effect of priming with a single dose of apomorphine on subsequent treatment with bromocriptine. Furthermore, we base much of the experimental design on the seminal paper by Gomez-Mancilla and Bedard (17), which defined the effects of several nondopaminergic drugs on L-DOPA-induced dyskinesia in the MPTP-treated nonhuman primate.

MATERIALS AND METHODS

Unilateral 6-Hydroxydopamine-Lesion of the Medial Forebrain Bundle

Male Sprague–Dawley rats (250–300 g) were obtained from Charles River (UK), maintained in standard housing conditions with constant temperature (22 ± 1°C), humidity (relative, 30%), 12-h light/dark cycles (light period 8 a.m./8 p.m.), and were allowed free access to food (Standard pellets, B & K Universal) and water. Thirty minutes prior to surgery animals were injected intraperitoneally (i.p.) with pargyline (5 mg/kg, Sigma) (to inhibit monoamine oxidase-B activity and thereby enhance the dopamine depletion by 6-hydroxydopamine) and desipramine (25 mg/kg, Sigma) (a noradrenaline uptake inhibitor to minimise damage to noradrenergic neurons) both dissolved in sterile 0.9% w/v sodium chloride (Braun Medical) and injected at a volume of 1 mg/kg of body weight. Rats were placed in a stereotactic frame, under sodium pentobarbitone anaesthesia (60 mg/kg, i.p., Sagatal, Rhone-Merieux). Each animal received a unilateral injection of 2.5 µl 6-hydroxydopamine HCl (Sigma, 5 mg/ml^{-1} in sterile water (Braun Medical) with 0.1% ascorbic acid (Sigma) into the right medial forebrain bundle at coordinates −2.8 mm from Bregma, 2 mm lateral to the midline, and 9 mm below the skull according to the atlas of Paxinos and Watson (30). The injection was made, by hand, over a 5-min period using a 5-µl Hamilton syringe. Postoperatively, all animals were placed on heated pads and received a subcutaneous injection of 12 ml sterile glucose-saline (0.18% w/v

sodium chloride, 4% w/v glucose, Intraven, IVEX Pharmaceuticals) to prevent postoperative dehydration.

Drug Treatment and Behavioral Analysis

Sterile water injection. Twenty-one days postoperation the 6-hydroxydopamine-lesioned rats were removed from their home cage, injected with sterile water (1 mg/ml^{-1}, i.p., Braun Medical) at 9 a.m., and placed immediately in a 40-cm-diameter stainless-steel bowl (day 0). Behavior was recorded on videotape for 3 h. Videotapes were analyzed by an observer blinded to the treatment, and the number of complete 360° rotations ipsiversive and contraversive to the lesion were counted over a 2-h period, immediately postinjection. 6- Hydroxydopamine-lesioned animals showing a net ipsiversive rotation less than 30 rotations in 2 h were removed from the experimental groups as previous studies have shown that such animals have a greater than 95% depletion in dopamine uptake sites as demonstrated by [^{3}H]mazindol binding autoradiography (unpublished observations).

Repeated dopamine-replacement therapy in the 6-OHDA-lesioned rat model of Parkinson's disease. Rats were injected intraperitoneally with either L-DOPA (L-3,4 dihydroxyphenylalanine methyl ester (Sigma), 6.5 mg/kg and 100 mg/kg), bromocriptine (2-bromo-α-ergocryptine (Sigma), 5 mg/kg), lisuride (R(+)-lisuride hydrogen maleate (Research Biochemicals International), 0.1 mg/kg), or vehicle (1 ml/kg) twice-daily at 9 a.m. and 5 p.m.

The methyl ester form of L-DOPA was administered, as it is more stable and soluble than nonesterified L-DOPA. L-DOPA methyl ester is rapidly deesterified *in vivo* by nonspecific esterases to form L-DOPA (13). In all experiments, L-DOPA was administered with 25 mg/kg benserazide (Sigma). All drugs were dissolved in 0.05% ethanol (BDH) and 0.1% ascorbic acid (Sigma) in sterile water (Braun Medical) and injected at a volume of 1 ml/kg of body weight). Vehicle consisted of 0.05% ethanol (BDH) and 0.1% ascorbic acid (Sigma) in sterile water (Braun Medical) and injected at a volume of 1 ml/kg of body weight.

Behavioral analysis was carried out as described above immediately following the 9 a.m. injection on days 1, 3, 5, 7, and 10 in rats treated with L-DOPA (100 mg/kg). In all other cases behavioral analysis was performed immediately following the 9 a.m. injection on days 1, 3, 5, 7, 10, 14, 17, and 21. Rats were removed from the stainless-steel bowls 3 h following the 9 a.m. injection.

A further study was performed to investigate the effects of priming with apomorphine on the response to subsequent bromocritpine treatment. Three weeks postoperation, 6-hydroxydopamine-lesioned rats were removed from their home cage and injected with either apomorphine hydrochloride (1 mg/kg, dissolved in sterile water, i.p., Sigma) or sterile water. Behavior was recorded on videotape, videotapes were analyzed, and the number of complete 360° rotations ipsiversive and contraversive to the lesion were counted over a 2-h period. Starting on the day following the apomorphine (or vehicle) challenge, rats received bromocriptine twice-daily, at 9 a.m. and 5 p.m. (5 mg/kg, i.p.). Following 21 days treatment behavioral analysis was carried out as previously described. Net rotations contraversive to the 6-OHDA-lesion were assessed, for 2 h, beginning 1 h following the 9 a.m. injection on day 21.

Effect of nondopaminergic drugs on L-DOPA-induced rotation in the 6-OHDA-lesioned rat model of Parkinson's disease following repeated L-DOPA treatment. Three weeks post 6-hydroxydopamine lesion, rats were treated with L-DOPA (6.5 mg/kg) and benserazide (1.5 mg/kg) for 14 days (twice-daily) as previously described to establish an enhanced level of response to L-DOPA. On days 15–21, treatment with L-DOPA was continued but additional treatment with nondopaminergic agents (or vehicle) was administered in concert with the 9 a.m. injection of L-DOPA. The nondopaminergic drugs were administered 40 min after L-DOPA. (Preliminary studies showed that the peak effect of L-DOPA occurred at 45 min postinjection (data not shown)). Rats were placed in stainless-steel bowls (diameter 40 cm) immediately following the L-DOPA injection and remained in the bowls for 3 h. Rotational locomotion was assessed for 15 min, between 45 and 60 min following L-DOPA injection. Injections of appropriate vehicle plus L-DOPA were made on the following day.

For each animal, net rotations, contraversive to the 6-OHDA-lesion were expressed as a percentage of the corresponding time period for the L-DOPA-sterile water treatment on the following day (percentage of hyperkinesia).

Drugs tested were the alpha$_2$-adrenergic receptor antagonist yohimbine (10 mg/kg, Sigma); the nonselective opioid receptor antagonist naloxone (0.03 mg/kg, Sigma); the alpha$_2$-adrenergic receptor agonist clonidine (1 mg/kg, Sigma); the 5-HT uptake inhibitor 5-methoxy 5-*N*,*N*-dimethyl-tryptamine (5-MDOT, 2 mg/kg, Sigma); the beta-adrenergic receptor antagonist and 5-HT1 agonist, propranolol (10 mg/kg, Sigma), all dissolved in sterile water (Braun Medical) and injected at a volume of 1 ml/kg. The animals were used for up to 4 nondopaminergic treatments with each second day as a vehicle control. Following 21 days of L-DOPA treatment the animals were killed by stunning and cervical dislocation. The brains were removed and rapidly frozen in isopentane cooled to −45°C. Brains were stored desiccated at −70°C until further processing.

Mazindol Radioligand Binding Autoradiography

To assess the extent of 6-OHDA-lesion dopamine uptake sites were assessed using [^{3}H]mazindol essen-

tially as we and others have previously described (18, 22). Brains were cryostat-sectioned (−19°C) at 15 μm, thaw-mounted onto gelatin/chrome-alum-coated slides and stored desiccated at −70°C until further processing. Sections were obtained from the striatum at a rostrocaudal level equivalent to +1.5 to +1.0 mm from bregma as defined by Paxinos and Watson (30). Sections were processed in triplicate. Sections were freeze-dried overnight. The sections were then preincubated in 50 mM Tris–HCl (pH 7.9) for 5 min at 4°C followed by 60 min incubation at 4°C in 50 mM Tris–HCl containing 300 mM NaCl, 5 mM KCl, 10 nM [^{3}H]mazindol (15–30 Ci/mmol, NEN/DuPont, UK), and 50 nM desipramine (RBI, U.S.A.). Specific binding was defined as that displaced by 100 μM nomifensine. Sections were then washed in ice-cold Tris–HCl (2 × 1 min), dip-rinsed in ice-cold distilled water, dried in a stream of cold air, and finally opposed to ^{3}H-sensitive film (Hyperfilm), alongside ^{3}H standards, for 2 weeks at 4°C. Films were developed using Kodak D-19 and fixed using Kodak Unifix. Average optical density of each striata and the standards was determined using a Seescan Image Analysis system and specific mazindol binding in pmol/mg determined. Only animals showing greater than 90% reduction in specific striatal [^{3}H]mazindol binding compared to the unlesioned side were included in the final analyses.

Spontaneous Locomotion Following Administration of Nondopaminergic Drugs in Normal, Nondopamine-Depleted Rats

To determine whether changes in rotation observed in 6-OHDA-lesioned rats following coadministration of L-DOPA with nondopaminergic were nonspecific changes in locomotion, rather than specific reductions in the enhanced responsiveness to L-DOPA, studies were performed to assess the effects of the drugs used on spontaneous locomotion of normal rats in an open field arena.

Twenty-four male Sprague–Dawley rats (250–300g) were obtained from Charles River (UK), maintained as before, and assigned to two groups, 12 vehicle-treated rats and 12 drug-treated rats. On day 1, both groups were injected with sterile water (1 ml/kg, i.p., Braun Medical, on day 1). Following a 5-min recovery period in their home cage, rats were placed in locomotion-monitoring cages consisting of perspex boxes 52 × 36 cm, with infrared detectors at 2.5-cm intervals (4 cm from the floor) along the perimeter (AM1051, Linton Instruments). The output of the infrared detectors was monitored by Amlogger software (Linton) for 30 min. Mobile counts, defined as the time in seconds that the rat is active, i.e., its central position changes by more than two grids in any 1 s, were recorded. On days 2, 4, 6, 8, and 10 nondopaminergic drugs were administered at the maximal doses tested in L-DOPA-treated 6-OHDA-lesioned rats. Rats were administered either drug or the appropriate vehicle and, following a 5-min recovery period in their home cage, were placed in the locomotion boxes. Drugs tested were yohimbine (10 mg/kg); naloxone (5 mg/kg); clonidine (1 mg/kg); 5-MDOT (2 mg/kg); propranolol (10 mg/kg), all dissolved in sterile water (Braun Medical) and injected at 1 ml/kg of body weight. On the day following each drug-trial day (i.e., days 3, 5, 7, 9, and 11), all rats were injected with sterile water (1 ml/kg, i.p., Braun Medical), and spontaneous locomotion (mobile counts) was assessed to ensure no drug or vehicle effects (on spontaneous locomotion) lasted more than 24 h.

Statistical Analysis

Rotational behavioral data, following repeated drug administration, were analyzed by one-way analysis of variance with repeated measures (ANOVA), followed by Student–Newman–Keuls test. The effects of apomorphine priming on 21-day bromocriptine administration were analyzed by one-way analysis of variance followed by Student–Newman–Keuls test. The effects of drug or vehicle administration on L-DOPA-induced hyperkinesia were analyzed using one-way analysis of variance (ANOVA) followed by Dunnett's test. Spontaneous locomotion following nondopaminergic drug or vehicle administration in normal, nondopamine depleted, rats were analyzed using unpaired Student's t test. The percentage reduction in specific [^{3}H]mazindol binding between drug-treated or vehicle-treated groups were compared using a one-way analysis of variance (ANOVA). In all tests significance was assigned when $P < 0.05$.

RESULTS

Lesion Assessment

In all of 6-hydroxydopamine-lesioned rats included in the study the specific mazindol binding on the lesioned side was less than 5% of the specific binding seen on the unlesioned side. No significant difference was observed in the percentage difference between lesioned and unlesioned side between any of the groups (ANOVA, $F_{4,29} = 0.59$, $P > 0.05$; Table 1). In all 6-OHDA-lesioned rats with this reduction in mazindol binding, injection of 1 ml/kg sterile water produced spontaneous rotation ipsiversive to the 6-OHDA-lesion immediately following injection (45 ± 2 ipsiversive rotations/2 h, $n = 30$).

Effect of Dopaminergic Drugs on Rotational Locomotion in the 6-OHDA-Lesioned Rat Model of Parkinson's Disease

Following a single injection of L-DOPA (100 mg/kg) and benserazide (25 mg/kg), rotation contraversive to

the 6-OHDA-lesion was observed (210 ± 52 contraversive rotations/2 h). With repeated treatment there was a significant difference in the rotational response to L-DOPA-administration (100 mg/kg, $F_{4,29} = 13.80$, $P < 0.0001$, ANOVA with repeated measures). Following repeated administration, this rotational response to L-DOPA showed plasticity, rising on days 3 and 5, and reaching a maximum by approximately day 7. The rotational response to L-DOPA was significantly different to day 1 on days 3, 5, 7, and 10 (day 3, $P < 0.05$; day 5, $P < 0.01$; days 7 and 10, $P < 0.001$, Student–Newman–Keuls test). By the end of the 10-day treatment period a 471% increase, compared to the first injection, in the rotational response to the same dose of L-DOPA/benserazide was observed (990 ± 92, Fig. 1).

Following a single injection of L-DOPA (6.5 mg/kg) and benserazide (1.5 mg/kg), no significant rotation contraversive to the 6-OHDA-lesion was observed (26 ± 7 ipsiversive rotations/2 h). With repeated treatment there was a significant difference in the rotational response to L-DOPA-administration (6.5 mg/kg, $F_{7,47} = 26.04$, $P < 0.0001$ ANOVA with repeated measures). A robust rotational response, contraversive to the 6-OHDA-lesion, was not seen until day 7 (267 ± 51). However, following a further 7 days of treatment, a rapid increase was observed following subsequent injections. This potentiated behavioral response reached a plateau by day 14 and by the end of the 21-day treatment period, a 334% increase in the rotational response is observed (892 ± 114, Fig. 1). The rotational response to L-DOPA was significantly different to day 1 on days 10, 14, 17, and 21 (day 10, $P < 0.01$, in all other cases $P < 0.001$, Student–Newman–Keuls test). Thus, twice-daily injection of the lower dose of L-DOPA (6.5 mg/kg) showed a more gradual potentiation in response to treatment than the higher dose requiring an additional 14 injections to cause a significant enhancement in rotational response (Fig. 1).

Following a single injection of vehicle, rotation ipsiversive to the 6-OHDA-lesion was observed (18 ± 7 ipsiversive rotations/2 h; Fig. 1). With repeated treatment there was no significant alteration in the rotational response to vehicle administration ($F_{7,47} = 1.92$, $P > 0.05$, ANOVA with repeated measures).

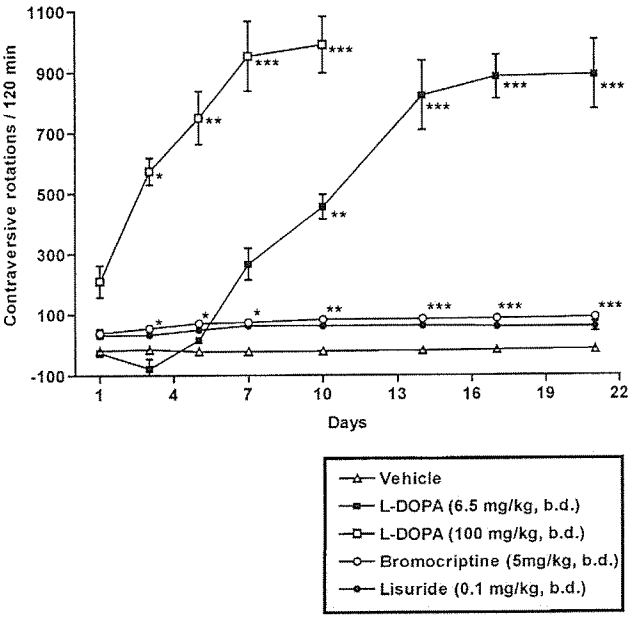

FIG. 1. Behavioral hyperkinesia following repeated L-DOPA administration in the 6-OHDA-lesioned rat model of Parkinson's disease. Net 360° rotations contraversive to the 6-OHDA-lesion were measured following twice-daily (9 AM and 5 PM) injections of L-DOPA (100 mg/kg) and benserazide (25 mg/kg, for 10 days), L-DOPA (6.5 mg/kg) and benserazide (1.5 mg/kg, for 21 days), bromocriptine (5 mg/kg, for 21 days), lisuride (0.1 mg/kg, for 21 days), or vehicle for 21 days in the 6-OHDA-lesioned rat model of Parkinson's disease. Locomotion was assessed for 2 h following the 9 AM injection on days 0, 1, 3, 5, 7, 10 for the high-dose L-DOPA and on days 0, 1, 3, 5, 7, 10, 14, 17, and 21 for the 6.5 mg/kg dose of L-DOPA, bromocriptine, lisuride, and vehicle. Data are expressed as mean (± SEM) net rotations contraversive to the 6-OHDA-lesion ($n = 6$, $^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$ compared to day 1 administration, repeated measures ANOVA, followed by Student–Newman–Keuls test).

TABLE 1

Percentage Reduction in Specific [^{3}H]Mazindol Binding in the Striatum Following 6-OHDA-Lesion ($n = 6$, $P > 0.05$, ANOVA, Student–Newman–Keuls Test)

6-OHDA-lesioned group	Percentage of reduction in specific [^{3}H]mazindol binding (mean ± SEM)
Vehicle	95.68 ± 1.65
L-DOPA (100 mg/kg)	98.24 ± 2.35
L-DOPA (6.5 mg/kg)	96.43 ± 1.65
Bromocriptine (5 mg/kg)	95.32 ± 1.12
Lisuride (0.1 mg/kg)	94.51 ± 1.12

Following a single injection of bromocriptine, rotation contraversive to the 6-OHDA-lesion was observed (38 ± 14 contraversive rotations/2 h; Fig. 1). With repeated treatment there was a significant difference in the rotational response to bromocriptine-administration (5 mg/kg, $F_{7,47} = 7.33$, $P < 0.0001$ ANOVA with repeated measures). The rotational response to bromocriptine was significantly different to day 1 on days 3, 5, 7, 10, 14, 17, and 21 (days 3, 5, 7, $P < 0.05$; day 10, $P < 0.01$; days 14, 17, and 21, $P < 0.001$, Student–Newman–Keuls test). On day 3 this rotational response increased (55 ± 8 contraversive rotations/2 h). However, bromocriptine administration (from day 3 to day 21) produced no further behavioral enhancement ($P > 0.05$, Student–Newman–Keuls).

Following a single "priming" dose of apomorphine (1 mg/kg) the response to 21-day bromocriptine (5 mg/kg) treatment was significantly different to "vehicle-primed" (Fig. 2, $F_{1,12} = 14.02$, $P < 0.0001$, ANOVA). Thus, on day 1 bromocriptine elicited 88 ± 41 contraver-

sive to the lesion in animals primed with apomorphine, this was not significantly different to the response to bromocriptine in "vehicle-primed" animals (52 ± 19, contraversive rotations/2 h, $P > 0.05$, Student–Newman–Keuls). However, following 21 days of treatment bromocriptine elicited 431 ± 68 rotations contraversive to the lesion (per 2 h) in animals primed with apomorphine, this was significantly different to the response to bromocriptine in "vehicle-primed" animals (95 ± 49 contraversive rotations/2 h, $P > 0.001$, Student–Newman–Keuls; Fig. 2).

Following a single injection of lisuride, rotation contraversive to the 6-OHDA-lesion was observed (31 ± 7 contraversive rotations/2 h; Fig. 1). With repeated lisuride treatment there was no significant alteration in the rotational response to lisuride-administration ($F_{7,47} = 2.21$, $P > 0.05$, ANOVA with repeated measures).

Effect of Coadministration of Nondopaminergic Drugs on L-DOPA-Induced Rotational Locomotion in the 6-OHDA-Lesioned Rat Following Repeated L-DOPA-Administration

Coadministration of nondopaminergic drugs, with L-DOPA, significantly altered the hyperkinesia induced by L-DOPA alone in rats that had received repeated L-DOPA treatment ($F_{5,37} = 78.24$, $P < 0.0001$, ANOVA). The alpha$_2$-adrenergic receptor antagonist yohimbine (10 mg/kg), the alpha$_2$-adrenergic receptor agonist clonidine (1 mg/kg), the 5-HT uptake inhibitor 5-MDOT (2 mg/kg), or the beta-adrenergic receptor antagonist propranolol (10 mg/kg) significantly reduced the L-DOPA-induced hyperkinesia (95, 93, 90, and 35% reduction, respectively, all $P < 0.01$, Dunnett's test; Fig. 3). In contrast, low-dose administration of the nonselective opioid-receptor antagonist naloxone (0.03 mg/kg) had no effect on the L-DOPA-induced hyperkinesia seen following repeated L-DOPA-administration in the 6-OHDA-lesioned rat (Fig. 3).

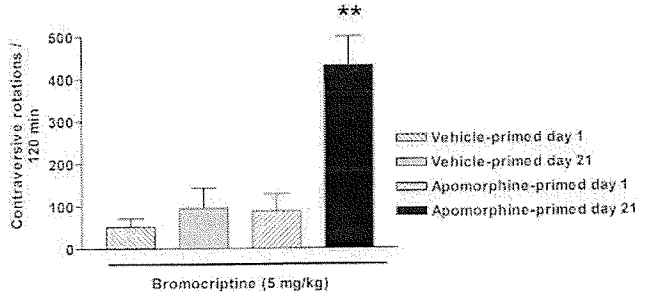

FIG. 2. Effects of apomorphine "priming" on rotational locomotion following repeated bromocriptine administration in the 6-OHDA-lesioned rat model of Parkinson's disease. Three weeks post 6-OHDA-lesioned rats were "primed" by a single injection of apomorphine (1 mg/kg) or vehicle. Starting on the following day bromocriptine (5 mg/kg) was administered twice-daily for 21 days. Net 360° rotations contraversive to the 6-OHDA-lesion are shown following the first administration on day 1 or following 21 days bromocriptine treatment in "apomorphine-primed" or "vehicle-primed" rats. Locomotion was assessed for 2 h following the 9 AM injection on day 21. Data are expressed as mean (± SEM) net rotations contraversive to the 6-OHDA-lesion ($n = 6$, $^{**}P < 0.01$ compared to "vehicle-primed" group, ANOVA, followed by Student–Neuman–Keuls test).

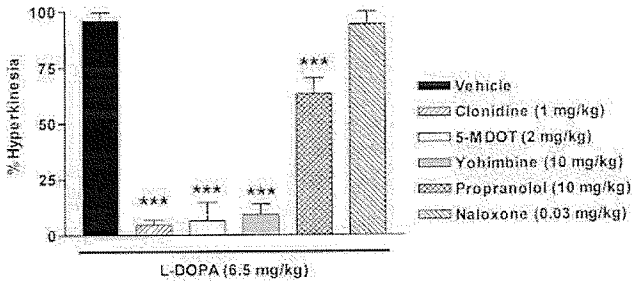

FIG. 3. Effects of coadministration of nondopaminergic drugs on L-DOPA-induced rotational locomotion in the 6-OHDA-lesioned rat following repeated L-DOPA administration. Following 14 days, twice daily, L-DOPA-administration in the 6-OHDA-lesioned rat, a behavioral hyperkinesia is observed. On days 14–21 rats were injected with L-DOPA (6.5 mg/kg) and benserazide (1.5 mg/kg). Net 360° rotations contraversive to the 6-OHDA-lesion were measured following L-DOPA (6.5 mg/kg) and benserazide (1.5 mg/kg) plus nondopaminergic drug or L-DOPA and benserazide plus vehicle and are expressed as a percentage of the next day vehicle. All drugs were administered to give maximal effect at 45 min following L-DOPA administration, the period previously determined as being the peak effect of L-DOPA. Locomotion was assessed for 15 min (45–60 min following L-DOPA administration). Data are expressed as mean (± SEM) percentage hyperkinesia ($n = 5$–8, $^{***}P < 0.001$ compared to vehicle administration, ANOVA, followed by Dunnett's test).

Effect of Nondopaminergic Drugs on Spontaneous Locomotion in Normal, Nondopamine-Depleted, Rats

To investigate the possibility that the reductions in hyperkinesia observed following coadministration of yohimbine, 5-MDOT, clonidine, and propranolol with L-DOPA in the 6-OHDA-lesioned rat were not due to nonspecific reductions in spontaneous locomotion, all drugs utilized in this study were administered to normal, non-dopamine-depleted rats and spontaneous locomotion was assessed. No significant effects on spontaneous locomotion were seen with either naloxone (1444 ± 283 mobile counts/30 min, vehicle for naloxone 1587 ± 222), propranolol (2356 ± 349 mobile counts/30 min, propranolol vehicle 2216 ± 400) or 5-MDOT (2200 ± 250 mobile counts/30 min, 5-MDOT vehicle 1739 ± 205) (in all cases $P > 0.05$, Student's t test). Yohimbine increased spontaneous locomotion by 213% (yohimbine vehicle 1177 ± 356, yohimbine 2517 ± 3556, mobile counts/30 min, $P < 0.01$, Student's t test). In contrast, clonidine produced an 80% decrease in spontaneous locomotion (vehicle 1302 ± 201, clonidine 300 ± 60, $P < 0.001$, Student's t test).

DISCUSSION

As previously described, 6-OHDA-injection into the median forebrain bundle produced a unilateral dopamine depletion characterized by greater than 95% reduction in striatal dopamine uptake sites, as assessed by [^{3}H]mazindol binding autoradiography. Following a single administration of L-DOPA (100 mg/kg) in the unilateral 6-OHDA-lesioned rat model of Parkinson's disease, a rotational response contraversive to the lesion was observed. This response is equivalent to the well-characterized contraversive rotation that has been much investigated since first described in the early 1970s (e.g., 38, 39). This response is generally held to reflect antiparkinsonian actions, indeed 2-deoxyglucose metabolic tracing experiments suggest that it is associated with changes in neural activity that would be consistent with such an effect (37).

However, following repeated treatment this response shows plasticity becoming markedly enhanced. Such enhancement is seen following injection of both doses of L-DOPA used in the present study. Similar enhancement of the rotational response to dopamine-replacement have previously been reported (5, 10, 16). It has been suggested that the enhanced response might be the result in changes in neural activity that are similar to those that underlie L-DOPA-induced dyskinesia in man and in the MPTP-treated primate. Indeed, the experimental paradigm models the precipitating factors for the production of L-DOPA-induced dyskinesia, i.e., dopamine depletion of the striatum and subsequent reintroduction in a pulsatile manner. However, as the behavior shows none of the complex movements, chorea for example, that are characteristic of dyskinesia in primates there was a need to further characterize this potentially important model.

It was thus of interest that, like dyskinesia, the enhanced rotational response to L-DOPA in 6-OHDA-lesioned rats is related to the dose and duration of treatment (28). The 6.5 mg/kg dose of L-DOPA and the bromocriptine and lisuride doses administered in this study were chosen on the basis of clinically relevant doses on a mg/kg basis (34, 35). In the 6-OHDA-lesioned rat, *de novo* administration of drugs that are associated with a lower incidence of dyskinesia in both patients and MPTP-lesioned nonhuman primates, i.e., bromocriptine (3) and lisuride (35), are associated with no, or markedly less, enhancement in response to repeated treatment. An initial enhancement in the behavioral response to bromocriptine was observed following 3 days administration, with no subsequent enhancement in the rotational response. This finding, although statistically significant, may result from the inherent variability in the initial rotational response to bromocriptine rather than a maintained enhancement of response. As noted above there is no further enhancement following further repeated treatment and the rotational response observed following bromocriptine administration in the vehicle-primed animals was similar (52 ± 19) to that seen following 3-day administration in the "nonprimed" group following repeated bromocriptine administration (55 ± 8). However, a behavioral potentiation is observed following repeated bromocriptine treatment after a single "priming" injection of the dopamine receptor agonist apomorphine (Fig. 2). In a similar manner bromocriptine elicits dyskinesia in animals that have previously been treated with dopamine-replacing agents that cause dyskinesia (4, 6). The fact that a single dose of L-DOPA can markedly affect the subsequent response to dopaminergic drugs also has major implications for the design of experiments assessing the actions of dopamine-replacing drugs as an apomorphine or L-DOPA challenge, to assess the extent of lesion, is a common feature of many experiments employing the 6-OHDA-lesioned rat.

Thus, although rats do not exhibit symptoms that can be described as dyskinesia, they do show an enhanced behavioral response to dopaminergic treatments that would elicit dyskinesia in primates. A further line of evidence to suggest that the neural mechanisms underlying this behavior are similar to those seen in L-DOPA-induced dyskinesia is provided by the study of the effects of nondopaminergic drugs on the actions of L-DOPA. Thus, we found that the alpha$_2$-adrenergic receptor antagonist yohimbine, the 5-HT uptake inhibitor 5-MDOT and the beta-adrenergic receptor antagonist propranolol reduced the enhanced response to L-DOPA. These effects did not reflect nonspecific reductions in locomotion, as they did not significantly reduce levels of spontaneous locomotion of normal rats in an open-field arena. The specific effects of these compounds on L-DOPA-induced rotation are very similar to those reported for the same three compounds on L-DOPA-induced dyskinesia in the MPTP-treated monkey (17). The data are also reminiscent of recent reports, suggesting that the alpha$_2$-adrenergic receptor antagonists idazoxan and rauwolscine can reduce dyskinesia in patients (32) and MPTP-lesioned monkeys (20), respectively. The finding that 5-MDOT reduces L-DOPA-induced rotation may reflect similar changes in neural activity to those underlying reports that the 5-HT uptake inhibitor fluoxetine can reduce apomorphine-induced dyskinesia in parkinsonian patients (15). Similarly, the beta-adrenergic receptor antagonist propranolol has also been reported to decrease L-DOPA-induced dyskinesia in a small-scale clinical trial (11).

A low dose of the nonselective opioid receptor antagonist naloxone had no effect on either the L-DOPA-induced rotation or dyskinesia. This finding is similar to that reported by Gomez-Mancilla and Bedard (17).

However, with respect to potential treatment for L-DOPA-induced dyskinesia, it is of interest to note

that it has previously been shown that, at higher doses, naloxone decreases L-DOPA-induced hyperkinesia (10, 19). The selective alpha-adrenergic receptor agonist clonidine reduced both L-DOPA-induced rotation in the present study and L-DOPA-induced dyskinesia in the study by Gomez-Mancilla and Bedard (17). However, these reductions affected locomotion generally, reducing both spontaneous locomotion in normal rats in the present study, and the antiparkinsonian effects of L-DOPA in primates (17).

The reductions in hyperkinesia observed by the various drugs tested in this study have been investigated at the time of peak L-DOPA effect. The potential of these findings with respect to the treatment of L-DOPA-induced dyskinesia clinically deserves further investigation. For instance, in this study neither the duration of action of these compounds nor any maintenance of reduction in hyperkinesia following repeated administration has been investigated.

Thus, the data presented in this paper suggest that the pharmacological profile of the potentiated response to repeated dopamine-replacement therapy in the 6-OHDA-lesioned rat is essentially identical to that of L-DOPA-induced dyskinesia in the MPTP-lesioned nonhuman primate model of Parkinson's disease. We propose that the mechanism underlying this behavior is similar to that seen in L-DOPA-induced dyskinesia in both patients and MPTP-lesioned nonhuman primates. This novel behavioral response may be usefully utilized to investigate the cellular and molecular mechanisms underlying symptom generation in patients and to provide an initial nonprimate screen to assess the potential of novel nondopaminergic drugs to elicit dyskinesia.

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